UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
NATIONAL CENTER FOR ENVIRONMENTAL ASSESSMENT
WASHINGTON, DC 20460

OFFICE OF
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To: Committee on Drinking Water Secretariat
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From: Lynn Flowers, PhD, DABT LF
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Re: Comments on Chromium in Drinking Water Document for Public Consultation (2015)

Dear CDW Secretariat:
Thank you for the opportunity to review the draft *Chromium in Drinking Water Document for Public Consultation*. US EPA's National Center for Environmental Assessment (NCEA) is actively engaged in an evaluation of the available literature regarding the potential health effects that may occur as a result of environmental exposure to hexavalent chromium. We are at the early stages of the development of a health assessment for the Integrated Risk Information System (IRIS) Program and released preliminary scientific materials in April and September 2014. The materials included a planning and scoping summary, problem formulation information, information on the literature search and screening strategy, the approach for selecting critical human and experimental animal studies, presentation of critical human and experimental animal data, exposure-response arrays, and a preliminary summary of pertinent toxicokinetic and mechanistic studies. We hosted two science meetings to provide an opportunity for the public to participate in an open discussion regarding the preliminary materials. This information can be found at http://www.epa.gov/iris/publicmeeting/iris_bimonthly-jun2014/index.htm and http://www.epa.gov/iris/publicmeeting/iris_bimonthly-oct2014/mtg_docs.htm. In addition, NCEA hosted a workshop in September 2013 to discuss the rates at which hexavalent chromium may be effectively detoxified in the gastrointestinal tract. Information on this workshop can be found at http://www.epa.gov/iris/irisworkshops/cr6/index.htm.

Our work to date has proved informative in our review of Health Canada's draft *Chromium in Drinking Water Document for Public Consultation*. Our comments focus on the draft hazard identification and dose-response information provided and are based on our own review of the literature thus far. NCEA has just begun to synthesize the available information and has not yet made any decisions regarding the final outcome of this analysis (e.g., decisions regarding whether and how mode of action (MOA) information informs the low-dose region of the dose-response curve for carcinogenic effects). Please find below general comments, followed by more specific comments on the oral rat tumors, MOA

analysis, and information regarding some of the cited references, and some information that may be useful regarding benchmark dose modeling. We hope that these comments are useful to Health Canada.

General comments

The draft document cites and quotes a particular subset of review articles to support the decision that hexavalent chromium is a threshold carcinogen. It appears that the totality of available data has not been evaluated. There are a number of original studies that do not appear to have been considered in the review articles, and as such are not represented in the draft document's evaluation and conclusions. The draft document also seems to provide contradictory evidence in support of a threshold MOA for oral and intestinal tumors. For example, a threshold approach for tumors of the mouse small intestine is proposed, based on evidence that hyperplasia occurred at doses lower those that induced tumor formation. However, the oral tumors were observed in rats, and hyperplasia was not observed in the rat oral cavity. The draft document would benefit from an evaluation of all of the available data, with a specific focus on how the mechanistic data inform both the oral and intestinal tumors in mice and rats. Specific comments are provided below for your consideration.

Oral rat tumors

The draft document cites a review by De Flora (2000) as the basis for determining a threshold mechanism for hexavalent chromium, including for oral tumors in rats. ("...De Flora (2000) concluded that experimental and epidemiological data point to the occurrence of thresholds in Cr(VI) carcinogenesis", page 52).

These tumors were highly invasive (in one instance, spreading to the brain), and rare (approximately 1% incidence in historical controls). There does not appear to be evidence in the NTP database that there were any precursors to these tumors. Although they occur at higher doses than the small intestinal tumors in mice, the draft document would benefit from a more thorough discussion of these data and how the available mechanistic data inform the low-dose region of the dose-response curve, including a discussion of the similarities and differences between rats and mice.

It should be noted that squamous cell carcinoma of the oral mucosa is not an endpoint that necessarily exhibits consistent site and species concordance across animal species, even for chemicals that are known to operate via a mutagenic MOA. This is similarly true for stomach/GI cancers. Thus, the lack of concordance in these tumors does not necessarily weaken or diminish their relevance to humans.

See references listed below for more details:

Sundeep A. Chandra, Michael W. Nolan, David E. Malarkey, (2010). Chemical Carcinogenesis of the Gastrointestinal Tract in Rodents: An Overview with Emphasis on NTP Carcinogenesis Bioassays, Toxicol Pathol. 38(1): 188–197

Tang, X. H., Knudsen, B., Bemis, D., Tickoo, S. and Gudas, L. J. (2004). Oral cavity and esophageal carcinogenesis modeled in carcinogen-treated mice. Clinical cancer research: an official journal of the American Association for Cancer Research 10(1 Pt 1), 301-13

Tsukamoto, T., Mizoshita, T. and Tatematsu, M. (2007). Animal models of stomach carcinogenesis. Toxicologic pathology 35(5), 636-48

The statement that "oral tumours do not appear to be relevant to humans" does not seem to be adequately supported by the arguments presented in section 9.3.2.

The statement that "Second, absorption of Cr(VI) to the oral cavity is effectively reduced" is confusing. Perhaps the intent was to use a word other than "absorption"?

The statement that "The transit time in the oral cavity is expected to be sufficiently short (few seconds) compared with that in the gastrointestinal tract..." is true, however reduction and uptake occur simultaneously. Since Cr(VI) uptake into cells is rapid, uptake may occur despite a short transit time. Most importantly, the transit time in the oral cavity of rats was also "sufficiently short", yet rats developed oral tumors.
The statement that "In addition, Cr(VI) is efficiently detoxified by saliva (De Flora, 2000)" deserves further attention. Drinking water may come into contact with the mouth and tongue before mixing with saliva, and uptake of Cr(VI) is possible. The draft document cites a study (De Flora, 2000) indicating that saliva in healthy human volunteers effectively reduced Cr(VI) in 5 minutes. However, a sample of five healthy adults would not be considered representative of the human population.

The statement that "Third, unlike the data for the small intestine, data for the oral cavity do not provide dose–response concordance" does not appear to be correct. The oral cavity is part of the rat digestive tract (thus supporting an overall species/organ system concordance). There is also a dose-response relationship. Some carcinogens that are known to produce oral/stomach/intestinal tumors in rodents do not exhibit site-concordance within the full GI tract across species. Refer to the articles listed previously (Chandra et al, 2010; Tsukamoto et al., 2007; Tang et al., 2004) for evidence of this.

Mode of Action Analysis

It appears that the MOA analysis in the draft document consists of summaries of select published review articles. For example, the text in Section 9.3 resembles the Thompson et al. (2013) review article (*Assessment of the mode of action underlying development of rodent small intestinal tumors following oral exposure to hexavalent chromium and relevance to humans*). The draft document would benefit from an independent, systematic evaluation of all of the available data. There are a number of original studies that do not appear to have been considered in the review articles, and as such are not represented in the draft document's evaluation and conclusions. An alternative approach would be to also discuss potential MOAs proposed by others (i.e., Witt et al. (2013), Collins et al. (2010), Zhitkovich (2011), and Stern (2010)), alongside the proposed MOA by Thompson et al. (2013). This would provide the reader with more of the available evidence and literature.

Specific comments on text:
Page 50:

> "Evidence for a mutagenic MOA is also weak. Hyperplasia was proposed to occur after DNA mutation (McCarroll et al., 2010). However, this MOA was based primarily on mutation data from non-target tissues, in vitro systems and mutations in K-ras/Apc genes that could not be replicated (McCarroll et al., 2010; O'Brien et al., 2013). Moreover, Thompson et al. (2013) believe that the high incidence (60%) of crypt proliferation after only 7days of Cr(VI) exposure is unlikely to be the result of a fixed mutation—especially considering the lack of neoplasms at day 90, the late tumour onset in the 2-year NTP mouse studies and the crypt hyperplasia in rats after

7 days of Cr(VI) exposure, but no hyperplasia or tumours after 2years (suggesting that hyperplasia is reversible and not the result of a fixed mutation)."

A critical analysis of these issues is recommended. For example, oral tumors were observed in rats, and hyperplasia was not observed in the oral cavity.

Page 51:

"The degree of confidence in the MOA is high".

This statement does not reflect the considerable uncertainties in the MOA for tumors of the small intestine. These uncertainties, and an alternative hypothesis, are outlined in Witt et al. (2013).

Page 51:

"Cr(VI) shares similar toxicological and carcinogenic characteristics with captan and folpet, which act via cytotoxic non-mutagenic MOAs".

Since Cr(VI) does not structurally resemble captan and folpet, is a metal, and induces oral tumors while captin and folpet do not. It appears that the MOA of captan and folpet may not be relevant to an assessment of Cr(VI).

Page 53:

"These BMDs/BMDLs were derived using the rodent PBPK model (Section 8.5) to estimate the amount of Cr(VI) entering each intestinal tissue section (duodenum, jejunum and ileum) from the lumen per day (normalized to intestinal tissue weight) in both sexes of mice."

The BMD modeling by Thompson et al. (2014) omitted hyperplasia data for the jejunum in the analysis. Thus, results are not based on all three intestinal tissue sections.

Page 53:

"...the data set for mouse small intestine hyperplasia is robust, based on 1500 data points (Thompson et al., 2014)"

It is unclear how there are 1500 data points. This number likely cites pharmacokinetic or other data points, which is not equivalent to dose-response data.

Page 52:

"Although the MOA for oral tumours in rats is not fully understood, De Flora (2000) concluded that experimental and epidemiological data point to the occurrence of thresholds in Cr(VI) carcinogenesis.."

As written, it appears that the draft document makes a major conclusion based on the position of a single review article authored by a single investigator in 2000. The reference could be considered obsolete on this topic, since it was published prior to the NTP (2008) bioassay. For additional reviews of the available literature see Witt et al. (2013), Collins et al. (2010), Zhitkovich (2011), and Stern (2010).

The lack of a MOA for oral tumors is a major uncertainty in the MOA for the small intestinal tumors. A more thorough review and analysis of oral tumors is recommended.

Section 9.3.1.3 (Lack of support for other carcinogenic MOAs):

It is recommended that a critical analysis of the mechanistic data, including additional studies that present alternative modes of action be included in this draft.

Furthermore, it is recommended that the calculation of an oral slope factor be done (both on the small intestinal tumors in mice, and the oral tumors in rats), even if it is only for comparative purposes.

Strengths or weaknesses of cited references

Throughout Section 9, studies with positive findings are summarily dismissed due to negative results in other studies, with no apparent consideration of what factors may have contributed to these discrepancies or how relatively important the different types of studies are (e.g., DNA strand breaks vs. unscheduled DNA synthesis). There is no evaluation of the quality of the toxicological studies or findings presented in the draft. While a weight of evidence framework is apparently applied and cited, it is recommended that the specific criteria that was used for weighing the impact of the available diverse study conclusions be included in the assessment.

Some specific comments regarding studies cited in the draft are noted below. This is not intended to be a complete list:

An erratum was published for the Kirman et al. (2013) study. The original PBPK model contained units and parameter errors regarding cardiac output and plasma/RBC reduction. Correcting these errors and re-fitting the model had little impact on estimates for absorption or reference values, indicating that the PBPK models may be over-parameterized. Schlosser and Sasso (2014) re-evaluated the data by Proctor et al. (2012) and present an alternative Cr(VI) gastric juice reduction model. It was determined that a threshold for stomach reduction in mice could not be ascertained from the data available at the time. Thus, the underlying whole-body PBPK model predictions for site-specific intestinal absorption have high uncertainties, particularly for the distal small intestine.

The paper by Lucaz et al. (2015) noted that some mechanistic data cited in Thompson et al. (2014) may not have detected genotoxicity due to the ascorbate/glutathione levels used in those experiments. (See Luczak et al., 2015). "Different ATM Signaling in Response to Chromium(VI) Metabolism via Ascorbate and Nonascorbate Reduction: Implications for in Vitro Models and Toxicogenomics.", Environ Health Perspect (in press)). As a result, there may be different interpretations of MOA studies cited by Thompson et al. (2014).

Additional strengths or weaknesses of the studies by Thompson et al. (2011-2013), O'Brien (2013), Kopec et al. (2012a,b), Kirman et al. (2012, 2013), Proctor et al. (2012) can be obtained from peer review reports on the following website: http://www.tera.org/Peer/Chromium/Chromium.htm.

Issues with several reproductive and developmental studies have been identified. The Junaid et al. (1996) reference, which studied effects in mice -

Junaid, M., Murthy, R.C., Saxena, D.K. (1996). Embryo- and fetotoxicity of chromium in pregestationally exposed mice. Bull Environ Contam Toxicol. 57: 327-334.

contains the same exact data as a separate reference by the same research group, apparently in rats -

Kanojia, RK; Junaid, M; Murthy, RC. (1996). Chromium induced teratogenicity in female rat. Toxicol Lett 89:207-213. (Not currently cited by Health Canada)

As a result, one cannot know which (if either) of these datasets is correct.

Two other references cited by the draft document (Banu et al. (2008) and Samuel et al. (2011)) also contain duplicated data. It appears that one of these studies may not have been conducted with a concurrent control (i.e., the same control groups are used as a comparison in both studies). In-fact, data from multiple exposure groups are identical in both papers. It also appears that some data may have

been incorrectly labeled, since identical results are presented in both papers but with different column headings.

BMD calculation

The draft document did not do BMD modeling of the mouse NTP data, but instead used the combined BMD/PBPK modeling results published in a journal article (Thompson et al., 2014). This does not allow for an independent verification of the modeling work. However, the cited report (Summit Toxicology, 2014) would add value to the analysis if it was included as part of an appendix.

For comparative purposes, BMD modeling results of the mouse and rat tumor data from NTP (2008) would also aid the assessment.

Miscellaneous

Information on Page 5 regarding the sources of Cr(VI) in the environment:

> "The principal source of Cr(VI) in the environment is anthropogenic pollution; Cr(VI) rarely occurs naturally due to its affinity for organic matter and other reducing substances (U.S. EPA, 1984c; Jaworski, 1985; Bartlett and James, 1988;Hammond, 2002).

More recent information shows that Cr(III) can transform into Cr(VI) as a function of geological conditions. A cursory literature search found Oze et al. (2007) and Kaprara et al. (2014) as studies on the conditions under which Cr(VI) can be formed. More references may be available:

Kaprara, E; Kazakis, N; Simeonidis, K; Coles, S; Zouboulis, AI; Samaras, P; Mitrakas, M. (2014). Occurrence of Cr(VI) in drinking water of Greece and relation to the geological background. Journal of hazardous materials. http://www.ncbi.nlm.nih.gov/pubmed/25085618

Oze, C; Bird, DK; Fendorf, S. (2007). Genesis of hexavalent chromium from natural sources in soil and groundwater. Proceedings of the National Academy of Sciences of the United States of America 104: 6544-6549. http://www.ncbi.nlm.nih.gov/pubmed/17420454, http://www.pnas.org/content/104/16/6544.full

The statement on page 35:

> "(4) support for exceedance of reducing capacity at ≥ 20 mg Cr(VI)/L (Proctor et al., 2012)."

While there is evidence for items 1-3 in the same paragraph, item 4 is still the subject of debate. This topic is further addressed in a peer-reviewed journal article that re-analyzed the data in Proctor et al. (2012) (see Schlosser and Sasso, 2014). This paper concludes that there is no well-defined threshold for Cr(VI) reduction in the gastric juice of rats or mice.

> Schlosser PM, Sasso AF. (2014). "A revised model of ex-vivo reduction of hexavalent chromium in human and rodent gastric juices". Toxicol Appl Pharmacol Oct 15;280(2):352-61.

The statements on Page 36:

> "Overall, the PBPK rodent model provides a good description of chromium toxicokinetics"

And:

> "Overall, the PBPK model provides a good description of chromium toxicokinetics and is consistent with the available total chromium data from Cr(III) and Cr(VI) exposures in typical humans."

As discussed in Schlosser and Sasso (2014), underlying kinetic models were not adequate descriptions of chromium toxicokinetics.

The statement on page 36:

> "3) that Cr(VI) enters portal circulation in rodents at drinking water concentrations ≥ 60 mg/L based on erythrocyte:plasma chromium ratios.."

Statements 1 & 2 are valid. However, erythrocyte:plasma ratios are not the only determinant of systemic absorption. Cr(VI) may be absorbed systemically but be reduced to Cr(III) in the GI tissue before accumulating in RBCs.

Suggestions for more concise text:

Section 9.1.4 and 9.2.4

Genotoxicity data summaries might be better summarized in a table.

Section 9.2.3

Study details and numbers might be better summarized in a table.

Section 9.3

All key events for the proposed MOA appear multiple times. The MOA information can be more succinctly presented (such as in tabular format and/or flow-chart format, alongside other potential MOAs).

Additional References

The following paper by the National Toxicology Program investigators outlines additional details, as well as new data analysis that was previously unpublished, regarding the NTP 2008 rodent bioassay:

> Witt, KL; Stout, MD; Herbert, RA; Travlos, GS; Kissling, GE; Collins, BJ; Hooth, MJ. (2013). Mechanistic Insights from the NTP Studies of Chromium. Toxicologic pathology 41(2): 326-42 http://www.ncbi.nlm.nih.gov/pubmed/23334696

The following paper contains more details regarding gastrointestinal tract tumors observed in the NTP database:

> Sundeep A. Chandra, Michael W. Nolan, David E. Malarkey, (2010). Chemical Carcinogenesis of the Gastrointestinal Tract in Rodents: An Overview with Emphasis on NTP Carcinogenesis Bioassays, Toxicol Pathol. 38(1): 188–197. doi:10.1177/0192623309356452.

The paper by Lucaz et al. (2015) examines the effect of ascorbate and glutathione on in-vitro genotoxicity estimates, which may be used to interpret some of the mechanistic data cited by Thompson et al. (2014):

> Luczak MW, Green SE, Zhitkovich A., (2015). "Different ATM Signaling in Response to Chromium(VI) Metabolism via Ascorbate and Nonascorbate Reduction: Implications for in Vitro Models and Toxicogenomics.", Environ Health Perspect (in press)